Oxidative damage and reduction of redox factor-1 expression after transient spinal cord ischemia in rabbits

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Objective: **The mechanism of spinal cord injury has been thought to be related to the vulnerability of spinal motor neuron cells against ischemia. However, the mechanisms of such vulnerability are not fully understood. We previously reported that spinal motor neurons may be lost by programmed cell death and thus now investigate a possible mechanism of neuronal death with immunohistochemical analysis for 8-hydroxy-2′-deoxyguanosine (8-OHdG) and redox factor-1 (Ref-1).**

Methods: **We used a rabbit spinal cord ischemia model with a balloon catheter. The spinal cord was removed at 8 hours, 1, 2, or 7 days after 15 minutes of transient ischemia, and histologic changes were studied with hematoxylin-eosin staining. Western blot analysis for Ref-1, temporal profiles of 8-OHdG and Ref-1 immunoreactivity, and double-label fluorescence immunocytochemical studies were performed.**

Results: **Most motor neurons were preserved until 2 days but were selectively lost at 7 days of reperfusion. Western blot analysis of a sample from sham control spinal cord showed a characteristic 37-kDa band that was reduced after ischemia. Immunohistochemistry showed the nuclear expression of Ref-1 in motor neurons of control spinal cords, and immunoreactivity was decreased 1 day after ischemia. On the other hand, no nuclear expression was seen of 8-OHdG in motor neurons of control spinal cords, and immunoreactivity was increased 1 day after ischemia. Double-label fluorescence immunocytochemical study revealed that both 8-OHdG and Ref-1 were positive at 8 hours of reperfusion in the same motor neurons, which eventually die.**

Conclusion: **These results suggest that Ref-1 decreased in motor neurons after transient spinal cord ischemia and that this reduction preceded oxidative DNA damage. The reduction of Ref-1 protein at the moderately late stage of reperfusion may be one of the factors responsible for the delay in neuronal death after spinal cord ischemia. (J Vasc Surg 2003;37: 446-52.)**

Motor neuron dysfunction from spinal cord injury after a successful operation of the thoracic aorta is a disastrous complication in humans. The reported incidence rates of paraplegia in such surgery range from 2.9% to 23%[1] in operations on the thoracic aorta. The cause of acute spinal cord dysfunction is believed to be ischemic damage during cross clamping. Ischemia can occur because of permanent exclusion of the essential intercostal arterial blood supply to the spinal cord or from temporary interruption of blood flow to the spinal cord.[2] However, patients who undergo thoracic aneurysm repair while awake with no neurologic deficit immediately after the operation may sometimes eventually have paraplegia develop, but this has been attributed to postoperative hypotension and low hemoglobin.[3,4] The exact mechanism of such delayed vulnerability is not fully understood. In a rabbit spinal cord ischemia model, we have reported delayed and selective motor neuron death after transient ischemia.[5,6] To evaluate the mechanism of such a delayed and selective vulnerability of motor neurons, we attempted to make a reproducible model for spinal cord ischemia and analyzed cell damage.

Many factors are involved in the development of spinal cord injury after ischemia; not only energy failure but also excitotoxcity, periinfarct depolarization wave oxidative stress, and alteration of gene expression play important roles in the pathogenesis of stroke.[7,8] Among these factors, the role of oxidative stress becomes much greater when spinal blood flow is restored because reflow to previous ischemic tissue results in an increase in oxygen level and consequently causes severe oxidative damage to the tissue with massive production of oxygen free radicals.[9]

In conditions of severe oxidative stress, the C-8 position of 2′-deoxyguanosine, which is the constituent of DNA, is hydroxylated and 8-hydroxy-2′-deoxyguanosine (8-OHdG) is produced.[10] Hydroxyl radical, singlet oxygen, and peroxynitrite are proposed to be responsible for this production.[11,12] Thus, it is currently considered that 8-OHdG is one of the best markers of oxidative DNA damage.[13] Aside from its role as an oxidative damage marker, 8-OHdG has the biologic significance of inducing

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Competition of interest: nil.

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G:C to T:A transversion.[14] It is postulated that this transversion may account for tumorigenesis of dividing cell origin.[13] On the other hand, this transversion in nondividing cells as neurons might be responsible for cell death, as many signals that culminate in carcinogenesis in dividing cells often cause cell death in neurons.[15] We have previously shown oxidative damage in neurons after cerebral ischemia in rats.[16] Thus, 8-OHdG production might be involved in the ischemia/reperfusion neuronal injury in the spinal cord after transient ischemia.

Apurinic/apyrimidnic (AP) endonuclease, or redox factor-1 (Ref-1), is a multifunctional enzyme involved in the base excision DNA repair of AP sites, which is a common form of DNA damage after oxidative stress.[17] The base excision repair pathway is initiated by removal of the damage base by a DNA glycosylase, which generates an AP site. Thereafter, Ref-1 cleaves the AP site 5¢ to phosphodeoxyribose, thus generating a free 3¢-hydroxyl necessary for DNA polymerase to fill the resulting gap.[18,19] The redox effector function of Ref-1 is to control the DNA binding of several transcription factors, such as Fos, Jun NFκB, and others.[20] The Ref-1 gene is activated by sublethal levels of reactive oxygen species, and expression of Ref-1 seems to correlate with cellular sensitivity to reactive oxygen species.[21] Recently, expression or activity of DNA repair enzymes, such as Ref-1 and 8-oxoguanine glycosylase/apyrimidinic/apurinic lyase, has been studied and has shown that Ref-1 is decreased in CA1 neurons and that this reduction precedes oxidative DNA damage after ischemic brain injury.[22,23] Therefore, we examined immunoreactivities of 8-OHdG and Ref-1 proteins after spinal cord ischemia for a possible involvement of apoptosis in this type of neuronal death.

MATERIALS AND METHODS

Animal models

During the experiment, the animals were treated in accordance with the declaration of Helsinki and the guiding principles in the care and use of animals. Also, the experimental and animal care protocol was approved by the animal care committee of Tohoku University School of Medicine.

Thirty Japanese domesticated white rabbits, weighing 2 to 3 kg, were used in this study and were divided into two subgroups: a sham control group and a 15-minute ischemia group. Anesthesia was induced with intramuscular administration of ketamine hydrochloride at a dose of 50 mg/kg and maintained with a 2% halothane inhalation. A 5F pediatric thermodilution catheter (405, B Braun Melsungen AG, Melsungen, Germany) was inserted through a femoral artery and advanced 15 cm forward into the abdominal aorta. Preliminary investigations with laparotomy confirmed that the balloon in the distal end of the thermodilution catheter was positioned 0.5 to 1.5 cm just distal to the left renal artery. During the experiment, aortic pressures were continuously monitored both at the proximal and distal positions of the balloon. Body temperature was monitored with a rectal thermistor and was maintained at 37° C with the aid of a heating pad during surgery and subsequent ischemia. The animals then were allowed free access of water and food at an ambient temperature. In the sham control, animals were killed just after insertion of the catheter into the abdominal aorta without inflation of the balloon. The two subgroups of animals were further divided into two experimental groups: group A, for investigation with histologic study (n = 18); and group B, for Western blot and immnohistochemical studies (n = 12).

Group A. The animals were allowed to recover at an ambient temperature and were killed with deep anesthesia of sodium pentobarbital (100 mg/kg intravenously) at 2 and 7 days after the reperfusion (n = 6 at each time point). For the six sham-operated controls, the animals were killed just after the insertion of the catheter into the abdominal aorta without inflation of the balloon. After death, spinal cords were quickly removed with the plunger of a 1-mL syringe. The samples for histologic study were fixed by immersion in 4% paraformaldehyde in 0.1 mol/L phosphate buffer and then stored at 4° C for 1 week; they then were cut transversely at about the L2 or L3 level and finally embedded in paraffin.

Group B. For Western blot analysis and immunohistochemical studies, the animals were killed at 8 hours, 1 or 2 days after blood flow restoration (n = 3 at each time point), and the samples were kept at −80° C until use. Sham control samples (n = 3) also were obtained.

Neurologic assessment

Neurologic function was observed at 2 days and at 7 days after the procedure. Animals were classified into a five-point scale according to the method of Johnson, Kraimer, and Graeber[24]: 0, hind-limb paralysis; 1, severe paraparesis; 2, functional movement, no hop; 3, ataxia, disconjugate hop; 4, minimal ataxia; and 5, normal function. Two individuals without knowledge of the treatment graded neurologic function independently. Statistical analyses of the neurologic score were done with the Mann-Whitney *U* test.

Histologic study

To see the pathologic changes of the spinal cord after ischemia, we performed hematoxylin-eosin staining with a set of sections and examined with light microscopy. An observer, unaware of animal group and neurologic outcome, examined each slide. With hematoxylin-eosin staining, the cells were considered "dead" if the cytoplasm was diffusely eosinophilic and "viable" if the cells showed basophilic stippling (that is, contained Nissl substance).[25] Statistical analysis of the cell number was performed with the Mann-Whitney *U* test.

Western blot analysis

To investigate changes of Ref-1 expression, we performed Western blot analysis. The tissue samples were homogenized in a lysis buffer (0.1 mol/L NaCl, 0.01 mol/L Tris-HCl, pH 7.5, 1 mmol/L ethylenediamine

tetraacetic acid, and 1 μg/mL aprotinin), and then the homogenates were centrifuged at 7000*g* for 15 minutes at 4° C. The supernatants were used as protein samples. Assays to determine the protein concentration of the supernatants were subsequently performed with comparison with a known concentration of bovine serum albumin with a kit (BCA protein assay reagent kit #23225, Pierce, Ill). Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was performed in a 10% polyacrylamide gel with nonreducing conditions. In brief, protein samples were boiled at 100° C in 2.5% SDS and 5% β-mercaptoethanol, and lysates equivalent to 20 _g of protein from each samples were run on the gel for 90 minutes at 20 mA, together with a size marker (rainbow colored protein, Amersham, Buckinghamshire, UK). The electrophoresis running buffer contained 25 mmol/L Tris base, 250 mmol/L glycine, and 0.1% SDS. The proteins on the gel then were transferred to a polyvinylidene fluoride membrane (Micron Separations Incorporation, Westboro, Mass) with a transfer buffer consisting of 48 mmol/L Trisbase, 39 mmol/L glycine, 0.4% SDS, and 20% methanol.

After the transfer, the membranes were placed in 1% powdered milk in phosphate-buffered saline solution (PBS) to block nonspecific binding. Then, they were incubated with primary antibody at 1:1000 dilution for 20 hours at 4° C. The primary antibody used was as follows: goat polyclonal anti-Ref-1 antibody (SC-9919, Santa Cruz Biotechnology, Inc, Calif). After washing in PBS, the membranes were incubated with biotinylated antigoat immunoglobulin-G (IgG) (PK-6105, Vector Laboratories, Burlingame, Calif) at 1:200 dilution in PBS for 90 minutes. They were washed in PBS and incubated with avidin-biotin-horseradish peroxidase complex (PK-6102, Vector Laboratories) for 60 minutes. The membrane then was developed with diaminobenzidine as a color substrate. The reaction was stopped with washing in distilled water. To ascertain specific binding of the antibody for the protein, another membrane was stained in a similar way without the primary antibody.

8-OHdG and Ref-1 immunocytochemistry

We performed an immunohistochemical study to investigate changes of expression of 8-OHdG and Ref-1. Spinal cord sections were rinsed in 0.1 mol/L PBS for 20 minutes and blocked in 2% normal horse serum for 2 hours at room temperature. Then, they were incubated with primary antibodies in 10% normal horse serum or 10% normal rabbit serum and 0.3% Triton-X 100 for 20 hours at 4° C, respectively. The primary antibodies used were as follows: mouse monoclonal anti-8-OHdG antibody (4355-MC-100, Trevigen, Inc, Gaithersburg, Md) and goat polyclonal anti-Ref-1 antibody (SC-9919, Santa Cruz Biotechnology, Inc). Each dilution was as follows: antibody against 8-OHdG at 1:200 and that against Ref-1 at 1:200.

After endogenous peroxidase activity was quenched with exposure of the slides to 0.3% H_2O_2 and 10% methanol for 20 minutes, the slides were washed in PBS and incubated for 3 hours with biotinylated antigoat IgG (PK-6105, Vector Laboratories) and biotinylated antimouse IgG (PK-6102; Vector Laboratories) at 1:200 dilution in PBS containing 0.018% normal horse and rabbit serum, respectively. Subsequently, the slides were incubated with avidin-biotin-horseradish peroxidase complex (PK-6102, Vector Laboratories). The slices were colorized with DAB/H_2O_2 solution. To ascertain specific binding of antibody for the protein, a set of sections was stained in a similar way without the primary antibody.

Fluorescence double-labeling study for 8-OHdG and Ref-1

Spinal cord sections were prepared as described previously. A nonspecific blocking procedure was performed with 10% horse serum before application of primary antibodies. Then, the sections were incubated with 8-OHdG mouse monoclonal antibodies 1:100 (Santa Cruz Biotechnology) simultaneously with Ref-1. These primary antibodies were incubated overnight at 4° C and were detected with donkey antimouse IgG linked with Texas Red 1:50 (715-075-147, Jackson Immunoreseach, Pa) and donkey antigoat lgG linked with fluorescein 1:50 (705-095-151, Jackson Immunoreseach). The slides were mounted in aqueous mounting media with DABCO and observed with fluorescein microscopy. We also examined the population of 8-OHdG and Ref-1 expressing cells. Data are presented as the mean ± the standard deviation of three rabbits.

RESULTS

When the balloon of the thermodilution catheter was inflated in the abdominal aorta, the systemic blood pressure of the rabbits did not change. The arterial pressure distal to the inflated balloon fell to near zero, and no pulsation was recorded. On deflation of the balloon, the systemic blood pressure of this portion decreased for 15 minutes and then returned to the normal level (data not shown). Spinal cord ischemia was achieved with the inflation of the balloon so as to stop blood flow to the spinal cord.[5,6]

Neurologic outcome. In the sham operation group (n = 6), all the rabbits were healthy (grade 5). In the 15-minute ischemia group at 2 days after the procedure (n = 6), three rabbits (50%) were healthy (grade 5), one rabbit (17%) had minimal ataxia (grade 4), and two rabbits (33%) had ataxia (grade 3). In the 15-minute ischemia group at 7 days after the procedure (n = 6), three rabbits (50%) did not hop (grade 2) and three rabbits (50%) had ataxia (grade 3). There was a significant difference between the Johnson neurologic scores at 2 days and at 7 days after the procedure in the 15-minute ischemia group (P = .0163). This difference was similar marked between the sham control group and the 15-minute ischemia group at 7 days after the procedure (P = .0039). Fifteen minutes of ischemia did affect neuronal function (Table I).

Histologic study. Representative photographs of hematoxylin-eosin-stained sections are shown in Fig 1 and Table II. After sham control, no significant change was seen in motor neurons (Fig 1 , *A*). After 15 minutes of ischemia on the day 7 of reperfusion, about 70% of motor neuron

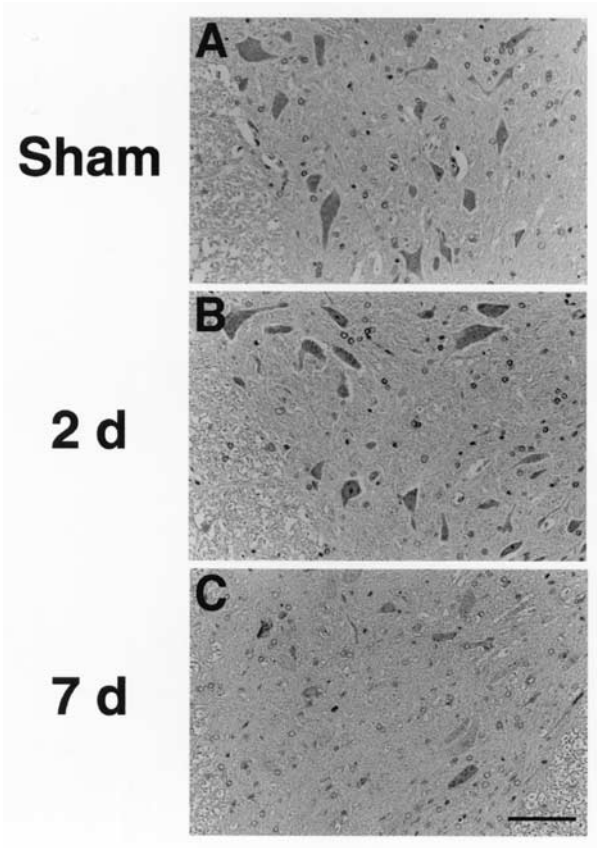

Fig 1. Histologic findings with hematoxylin-eosin staining of spinal cord after 15 minutes of ischemia. Spinal cord of sham control **(A)** and that at 2 days after ischemia **(B)** showed no histologic changes. At 7 days after ischemia **(C)**, however, motor neurons were selectively lost, without apparent gliosis or cellular infiltration. *Bar,* 100 μm.

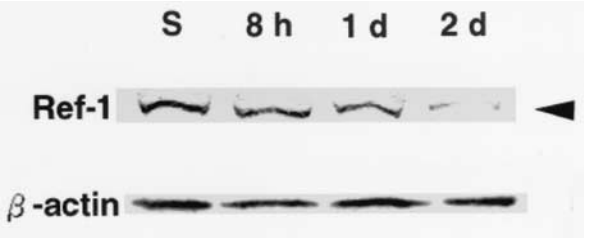

Fig 2. Representative Western blot for Ref-1 *(upper)* and β-actin *(lower)*. Ref-1 immunoreactivity was evident as single band (*upper;* sham, 8 hours, and 1 day), and band of Ref-1 was almost lost at 2 days. β-Actin showed no change.

cells were lost (Fig 1, *C*), although most motor neuron cells had remained intact after 2 days of reperfusion ($P = .0104$). Small motor neurons and intermediate neurons survived the ischemia (Fig 1, *B*). Dorsal horn neurons were intact after 15 minutes of ischemia at any time point (data not shown). The results of cell counting in the ventral gray matter region on the paraffin sections obtained from another series of animals are shown in Table II. The 15-minute ischemia at 7 days after the procedure affected the number of motor neuronal loss cells in contrast to sham control ($P = .0039$). Thus, selective loss of motor neurons was confirmed, in accordance with our previous reports.[5,6]

Western blot analysis. Representative results of Western blot analysis are shown in Fig 2 and Table III. With antibody against Ref-1, immunoreactivity was evident as a single band of 37 kDa was detectable in samples of sham control, and those at 8 hours and 1 day after blood flow restoration revealed a single band. This band became scarcely detectable at 2 day after reperfusion. In contrast, a consistent amount of β-actin immunoreactivity between each lane was shown in the lower panel, suggesting that the amount of the loaded protein was consistent. The membrane without the primary antibody revealed no band (data not shown).

Histochemical study. Immunoreactive 8-OHdG and Ref-1 of sections from spinals cords are shown in Fig 3. Motor neuron nuclei selectively showed strong immunoreactivity for Ref-1 (Fig 3 , *A to C*) antigen at sham, 8 hours, and 1 day of reperfusion after 15 minutes of ischemia. Immunoreactivity of motor neuron nuclei for Ref-1 (Fig 3 , *D*) antigen was almost lost in the motor neurons at 2 days. The spinal cords of sham-operated animals did not show 8-OHdG (Fig 3 , *E*) immunoreactivities in any cells. The samples of 8 hours of reperfusion also did not show 8-OHdG (Fig 3 , *F*) immunoreactivities in any cells. Motor neuron nuclei selectively showed strong immunoreactivity for 8-OHdG (Fig 3, *G, H*) at 1 day and 2 days of reperfusion.

Fluorescence double-labeling study. The results of 8-OHdG and Ref-1 double-staining immunohistochemistry are shown in Fig 4. 8-OHdG was strongly colocalized with Ref-1 in motor neurons at 1 day of reperfusion. Furthermore, most of the motor neurons expressed both 8-OHdG and Ref-1 (Table IV).

DISCUSSION

We have shown delayed and selective motor neuron death in lumbar regions of the rabbit spinal cord with a reproducible model. The histologic patterns of 15-minute ischemia in our model are reproducible. Analysis of immunohistochemical study for 8-OHdG and Ref-1 were also reproducible at each time point.

We have previously shown delayed and selective motor neuron death in lumbar regions of the rabbit spinal cord with the same reproducible model.[5,6] Fifteen minutes of ischemia is a relatively short period in comparison with those of previously reported ischemic models. After the ischemia, delayed and selective motor neuron damage was observed only after 7 days of reperfusion, a phenomenon known as selective neuronal death in motor neuron cells after spinal cord ischemia,[5,6,26] similar to the delayed selective neuronal death in hippocampal CA1 cells after cerebral ischemia.[27] Despite restoration of blood flow,[28] motor neurons, which initially appear to have survived ischemic

Table I. Neurologic score at 2 days and 7 days after procedure

Animal no.	*Sham control at 7 d*	*15-Min ischemia*	
		At 2 d	*At 7 d*
1	5	5	3
2	5	3	2
3	5	5	2
4	5	5	3
5	5	4	2
6	5	3	3
Mean ± standard deviation	5 ± 0*	4.2 ± 0.983†	2.5 ± 0.548

Compared with 15-minute ischemia group at 7 days after procedure:
*$P = .0039$.
†$P = .0163$.

Table II. Numbers of large motor neurons at 2 days and 7 days after ischemia

Treatment of animals	*Date*	*No.*	*Cell numbers*
Sham		6	20.7 ± 2.944*
15-Min ischemia	2 d	6	18.7 ± 4.633†
	7 d	6	8.2 ± 2.563

Values are mean ± standard deviation.
Compared with 15-minute ischemia group at 7 days after procedure:
*$P = .0039$.
†$P = .0104$.

Table III. Immunoreactivity for 8-OHdG and Ref-1 in motor neuron nuclei after ischemia

	8-OHdG	*Ref-1*
Sham control	−	2+
	−	2+
	−	2+
8 h	−	2+
	−	2+
	−	2+
1 d	2+	2+
	2+	2+
	2+	+
2 d	2+	−
	2+	−
	2+	−

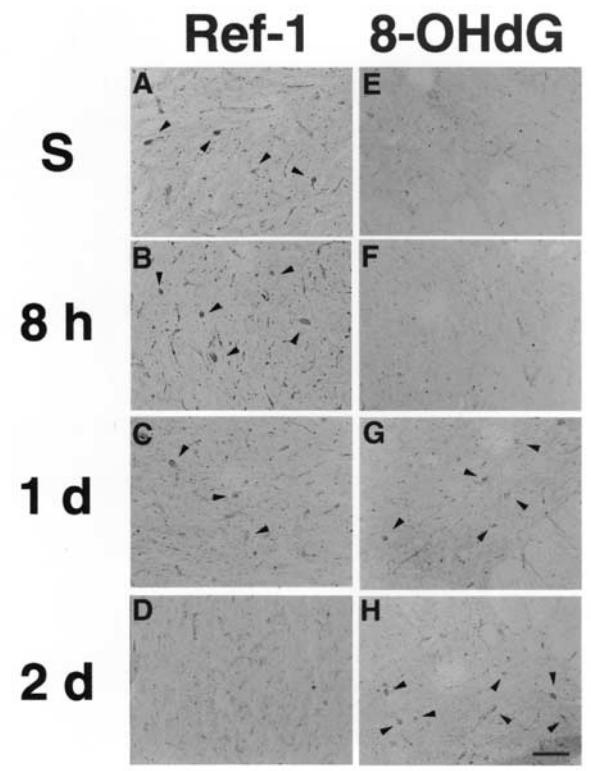

Fig 3. Immunostaining against Ref-1 and 8-OHdG in motor neurons in sham spinal cord **(A, E)** and at 8 hours **(B, F)**, 1 day **(C, G)**, and 2 days **(D, H)** of reperfusion. *Arrowheads* show motor neuron nuclei that express immunoreactive Ref-1 **(A, B, C)** and 8-OHdg **(G, H)**, respectively. *Bar,* 100 μm.

insult, go on to die days later. This result shows that motor neuron cells are most vulnerable to spinal ischemic injury.

Many recent studies on ischemic cerebrovascular disease have revealed that there are two major ways for neuronal cell death to occur—necrosis and apoptosis.[29] Necrosis usually occurs after acute ischemia from a reduction in the tissue ATP level accompanied by edema formation and concomitant death of glial and vascular cells. In contrast, neuronal apoptosis occurs days after ischemia without a reduction in the ATP level, nor edema formation and concomitant death of glial and vascular cells. We have previously reported that delayed and selective motor neuron death in this rabbit model may indicate apoptotic change.

Severe oxidative stress causes various kinds of damage in cells but the damage on DNA is of particular relevance.[30] This includes base and sugar lesions, single strand breaks, abasic site formation, and DNA-protein cross links.[31] Among these, 8-OHdG is considered one of the DNA oxidation byproducts. It is generated in cells with oxidative damage but not in those with nonoxidative damage.[13] Therefore, it is one of most popular markers of oxidative damage. This study clearly showed that only neurons produced immunoreactive 8-OHdG in the spinal cord, indicating that neurons are specifically subjected to oxidative DNA damage. This might be because excitotoxic mechanisms are involved in oxygen radical formation.[32] Because the glutathione level reduced is relatively low in neurons, nonneuronal cells dodged oxidative damage with a high level of glutathione reduced form but neuronal cells did not.[33]

Because 8-OHdG itself induces tranversion of G:C to T:A,[20] 8-OHdG might result in impairment of normal cell function with this transversion for cells that evaded acute lethal cellular injury. It is reported that rats subjected to the

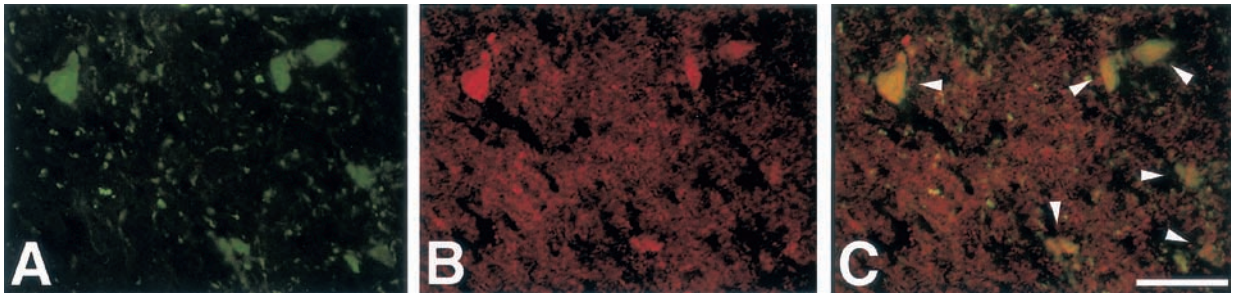

Fig 4. Colocalization of Ref-1 and 8-OHdG in motor neuron nuclei at 8 hours after ischemia. Ref-1 was detected with fluorescein isothiocyanat (*green*; **A**), and 8-OHdG with Texas red (*red*; **B**). Merged image is shown in **(C)**, with double positive as yellow color. *Bar,* 50 μm.

Table IV. Colocalization of 8-OHdG and Ref-1 in spinal motor neurons

	No. of motor neurons (mean ± standard deviation)
Ref-1 positive	15.0 ± 2.6
8-OHdG positive	16.3 ± 2.1
Ref-1 and 8-OHdG positive	14.3 ± 2.1

ferric nitrilotriacetate treatment, which induces 8-OHdG production in the kidney, are prone to renal cell carcinoma.[34] We have previously reported that 8-OHdG is produced in neurons after transient cerebral ischemia, which show apoptotic change and eventually die. Therefore, our results suggest an apoptotic change in motor neurons after transient spinal cord ischemia.

In the DNA repair mechanism, Ref-1 is drawing particular attention because of its critical role in the redox regulation of DNA-binding activity of the activator protein-1 family members, such as Fos and Jun transcription factors,[34,35] which are also considered to be associated with the pathogenesis of cerebral ischemia.[36] However, recent in vivo studies showed a lack of correlation between Ref-1 protein levels and expression of inducible transcription factors c-fos and c-jun, suggesting that Ref-1 protein is more likely to be involved in the repair of spontaneous DNA damage than posttranslational modification.[37] Furthermore, an increase in mRNA expression and a decrease in Ref-1 protein level after transient forebrain ischemia in rats have been reported.[38] On the other hand, Walto et al[39] showed the elevation of c-jun expression at the same time as a decrease in Ref-1 expression after hypoxic-ischemic insult in neonatal rat brains, suggesting the possibility that the loss of Ref-1 protein expression is not from a nonspecific decrease in protein synthesis. In addition, these cells were not fully compromised and retained at least metabolic activity. These results support the hypothesis that the reduction of Ref-1 is involved in the active process of programmed cell death.

Although the mechanism of Ref-1 reduction after reperfusion is not clearly known, mitochondrial production of superoxide radicals[40] could reduce the expression of Ref-1. Ref-1 has been described as playing a central role in base excision repair by 3′-OH primer for synthesis of DNA after all types of oxidative damage.[41] Because our results showed that 8-OHdG was strongly colocalized with Ref-1 in motor neurons, reduction of Ref-1 is one of the factors responsible for the delay in neuronal death after spinal cord ischemia.

A recent study showed that the protein levels of Ref-1 progressively decreased during the presymptomatic stage in transgenic amyotrophic lateral sclerosis model mice.[42] In this study, the decrease in the immunoreactivity of Ref-1 was shown selectively in ventral motor neuron cells in the spinal cord after 1 day of reperfusion. This finding suggests that an early impairment of DNA repair in the spinal motor neurons may account for the mutant superoxide dismutase-1–mediated motor neuronal death. Therefore, our results suggest that the mechanism of motor neuron death in the spinal cord after ischemia might have a similar feature with that of amyotrophic lateral sclerosis.

This study also showed that immunoreactivities for both 8-OHdG and Ref-1 were induced at 1 day in the same motor neurons that eventually die. The reduction of Ref-1 protein at the moderate late stage of reperfusion may be one of the factors responsible for the delay in neuronal death after spinal cord ischemia.

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